A comparison of the responses of *Tetranychus urticae* (Acari: Tetranychidae) and *Phytoseiulus persimilis* (Acari: Phytoseiidae) to volatiles emitted from lima bean leaves with different levels of damage made by *T. urticae* or *Spodoptera exigua* (Lepidoptera: Noctuidae)

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(Received 2 October 2002; Accepted 25 November 2002)

**Abstract**

Volatile compounds from lima bean leaves heavily infested with the herbivorous spider mite, *Tetranychus urticae*, attracted the carnivorous mite, *Phytoseiulus persimilis*, and repelled *T. urticae* over volatiles from uninfested leaves. Significantly more herbivore-induced volatiles were emitted from heavily infested leaves than from slightly infested leaves. When leaves were moderately infested, the amount of induced volatiles was intermediate between those of heavily infested and slightly infested leaves. These volatiles attracted *P. persimilis*, but neither attracted nor repelled *T. urticae* more than uninfested leaves. When leaves were slightly infested with *T. urticae*, small amounts of induced volatiles were detected. These volatiles neither attracted nor repelled *P. persimilis* more than uninfested leaves, but attracted *T. urticae* more than uninfested-leaf volatiles. The volatiles from lima bean leaves that were slightly or heavily infested with the herbivorous lepidopteran, *Spodoptera exigua*, neither attracted nor repelled *T. urticae* or *P. persimilis*. Most of the volatile compounds emitted from leaves heavily-infested with *S. exigua* were the same as those emitted from *T. urticae*-infested leaves, but the blend of the volatiles differed with the infesting species. The functions of volatiles emitted from herbivore-infested leaves are discussed in terms of herbivore density and their specificity.

**Key words:** Lima bean; *Tetranychus urticae*; *Spodoptera exigua*; *Phytoseiulus persimilis*; Y-tube olfactometer

**INTRODUCTION**

In many tritrophic systems consisting of plants, herbivores and carnivores, herbivore-infested plants emit so-called ‘herbivore-induced plant volatiles’ that attract carnivores (reviewed by Takabayashi and Dicke, 1996; Dicke, 2000). For example, in the tritrophic system consisting of *Phaseolus lunatus* (lima bean plants), the herbivorous spider mite, *Tetranychus urticae*, and the carnivorous mite, *Phytoseiulus persimilis*, the infested plants emit *T. urticae*-induced plant volatiles that attract *P. persimilis* (reviewed by Dicke et al., 1990; Takabayashi and Dicke, 1996). In contrast to accumulating studies on the response of predators to such induced volatiles, little is known about the response of herbivores to volatiles emitted from plants infested by herbivores (Dicke, 1986; Hildebrand et al., 1993; Pallini et al., 1997; Bernasconi et al., 1998; Shiojiri et al., 2002). Dicke (1986) first reported that *T. urticae* dispersed from volatiles emitted by lima bean leaves that were infested with conspecific mites. Conversely, *T. urticae*-infested cucumber leaves attracted conspecifics (Pallini et al., 1997).

The production of herbivore-induced volatiles is expected to be affected by the density of herbivores on a plant. However, there are few studies that focus on such effects (e.g. Maeda and Takabayashi, 2001). *T. urticae* is a tiny herbivorous mite (ca. 0.5
mm). Its population size increases rapidly on plants in the absence of carnivores, such as predatory mites. Using a Y-tube olfactometer, Sabelis et al. (1984) reported a significantly positive response by the predatory mite, *P. persimilis*, to volatiles from leaves infested with *T. urticae* of all feeding stages (larva, protonymph, male deutonymph, adult male) when the number of feeding mites was sufficiently high. Maeda and Takabayashi (2001) showed that the intensity of olfactory responses by *P. persimilis* to induced volatiles from kidney bean plants correlated with the amount of volatiles that followed an increase in the number of *T. urticae* on the plant. In contrast, Dicke (1986) reported that *T. urticae* females dispersed from the volatiles emitted by conspecific-infested leaves. To our knowledge, a detailed comparative study on the effects of the number of spider mites per leaf on the response of both predatory and spider (herbivorous) mites under the same conditions has not yet been carried out. Such a study is important for evaluating the ecological function of herbivore-induced plant volatiles.

Plants are naturally infested with several herbivore species. When a non-prey species of *P. persimilis* infests a plant, the plant emits non-prey induced volatiles, and this may affect the behavior of *P. persimilis* and *T. urticae* (e.g., Sabelis et al., 1999). Shimoda and Dicke (1999) reported that lima bean leaves infested with the lepidopteran *Spodoptera exigua* affected the olfactory response of *P. persimilis*. However, the response of *T. urticae* to those volatiles has not been tested.

In this paper, we focus on the density effects of *T. urticae* on volatile production in lima beans. We used a Y-tube olfactometer to observe the responses of *P. persimilis* and *T. urticae* to lima bean leaves infested with a varying number of *T. urticae* and compared these responses to those of uninfested leaves. Further, we compared the response of *T. urticae* and *P. persimilis* to volatiles from uninfested lima bean leaves with those infested with low and high densities of *S. exigua* larvae. To compare the qualitative and quantitative differences in volatiles emitted from lima bean leaves infested with *T. urticae* or *S. exigua*, we chemically analyzed the volatiles. Based on our behavioral and chemical data, the multifunctional aspects of herbivore-induced plant volatiles are discussed.

**MATERIALS AND METHODS**

**Plants and herbivores.** Lima bean plants, *Phaseolus lunatus* cv. Sieva, were separately grown in 12×10 cm plastic pots in a climate-controlled room (25±2°C, 50–70% R.H.). Two to three-week-old potted plants, with two primary leaves, were used in the experiments. Populations of the herbivorous spider mite, *T. urticae*, were obtained from a laboratory-maintained culture and reared on kidney-bean plants, *P. vulgaris* cv. Nagazuramame. The plants had been grown under the same conditions as the lima bean plants.

Populations of the herbivorous lepidopteran, *S. exigua*, which is not a prey species of *P. persimilis*, were obtained from a culture kept at the Agro-science Research Laboratory, Sankyo Co. Ltd. Eggs were kept in the climate-controlled room, as described above. Newly hatched first-instar larvae were used in the experiments.

**Infestation of lima bean leaves with *T. urticae.*** A primary leaf and its petiole were detached from each young plant. The petiole was soaked in a 6 ml vial that was filled with distilled water. To each leaf, 20, 100 or 300 adult female *T. urticae* were added. The leaf was then placed in the climate-controlled room (25±2°C and 16L–8D conditions, 2,150 lx, fluorescent lights) for 24 h. Only recently molted female *T. urticae* were used. After 24 h, each leaf was categorized by the number of mites as slightly, moderately or heavily infested.

**Infestation of lima bean leaves with *S. exigua.*** A primary leaf was put into a vial, as above. Two or 10 first instar nymphs were added to each leaf and placed in the climate-controlled room for 24 h. The leaves were then categorized as slightly or heavily infested.

**Bioassays using a Y-tube olfactometer.** A Y-tube olfactometer was used to test the olfactory responses of adult female *T. urticae* and their adult female predator, *P. persimilis*, to plant volatiles (for details of the olfactometer, see Takabayashi and Dicke, 1992). Bioassays were performed at 23±2°C. For each experiment, five treatment and five uninfested control leaves were compared. Bioassays of the leaves of treatments (1) to (3) (see below) vs. uninfested leaves, and the leaves of (4) and (5) vs. uninfested leaves, were carried out on the same experimental day. The bioassays were carried out over four experimental days [n=82...
treatments for (1) to (3) and 60 treatments for (4) and (5)]. The odor sources were renewed each experimental day. The treatments of each experiment were:

(1) slightly infested with *T. urticae* (20 *T. urticae* per leaf)
(2) moderately infested with *T. urticae* (100 *T. urticae* per leaf)
(3) heavily infested with *T. urticae* (300 *T. urticae* per leaf)
(4) slightly infested with *S. exigua* (2 *S. exigua* per leaf)
(5) heavily infested with *S. exigua* (10 *S. exigua* per leaf)
(6) A set of *T. urticae* (100 or 1,500 females), their associated products such as feces, silk and exuviae from five slightly and heavily infested leaves, and five uninfested leaves vs. five uninfested leaves, only.

Adult female *P. persimilis* individuals were starved for 24 h before the experiments commenced. They were individually positioned on the starting point of an iron Y-shaped wire that was fixed at the center of the Y-tube. When a mite reached one end of the wire arm ‘choice’ was recorded. However, when a mite did not reach the end within 5 min ‘no choice’ was recorded. After every fifth bioassay, we exchanged the arm containing the sample odor with an arm containing the control odor. Sabelis et al. (1984) reported *T. urticae* did not attract *P. persimilis*, and their feces only slightly attracted *P. persimilis*. We did not test the response of *P. persimilis* toward odor source treatment (6) in this study.

The olfactory responses of adult-female *T. urticae* were also tested. Two hours before the experiments, kidney-bean leaves heavily infested with *T. urticae* were put into a 12×6 cm plastic cup. Females started dispersing from the leaves and began walking along the sides of the cup. We collected the dispersed mites with a fine brush for bioassay. These females were individually positioned on the starting point of the iron Y-shaped wire that was fixed at the center of the Y-tube. The sample sizes and methods were the same as for *P. persimilis*, described above. In addition, the Y-shaped wire was cleaned with a piece of moist cotton wool after each assay to remove any spider webs left on the wire.

The data were analyzed with a chi-square test to test the null hypothesis that herbivore and carnivore had a 50:50 distribution across the two odor sources. Furthermore, the data were subjected to a contingency table analysis with a chi-square test to assess the effects of two or three herbivore-density treatments. When there was a significant difference between the attraction of the herbivore or carnivore for the three herbivore densities, each combination of two densities was tested with a 2×2 contingency table analysis, using the Bonferroni approach (*p=0.05/3=0.016*).

**Chemical analysis of volatile compounds**. Two treated leaves were placed in an airtight 2 l glass bottle for 30 min. The volatiles were collected from the bottle using 100 mg of Tenax TA resin (20/35 mesh; GL Science Inc., Japan) packed in a glass tube (3 mm i.d.×160 mm length) for 1 h, at a flow rate of 100 ml/min. The volatiles trapped on the Tenax TA were eluted with 2 ml of diethyl ether, and 0.5 μg of n-eicosane was added to the eluate as an internal standard. The eluate was concentrated with a stream of gaseous N2 and injected into the injection port (250°C) of a gas chromatography-mass spectrometer (GC: Hewlett-Packard 6890 with an HP-5MS capillary column, 0.25 mm i.d.×30 m length, 0.25 μm film thickness; MS: Hewlett-Packard 5973 mass selective detector, 70 eV). The GC oven temperature was programmed to rise from 40°C (5 min hold) to 280°C at 15°C/min. We assessed the levels of herbivore-induced volatiles, which are defined as the volatiles found in the headspace of treated leaves but which were absent, or in trace amounts, in those of untreated leaves. The chemical structure of each compound was determined by comparing the mass spectra and retention time with those of authentic chemical data. Each volatile compound emitted from leaves with varying infestation levels of *T. urticae* or *S. exigua* was compared to the control volatiles using a one-way ANOVA followed by Scheffe post hoc test.

**RESULTS**

**Responses of *T. urticae* to the volatiles from conspecific or *S. exigua*-infested leaves**

Significantly fewer *T. urticae* moved toward the volatiles of leaves that were heavily infested with conspecifics than toward uninfested, control leaves (Fig. 1A). In contrast, more *T. urticae* moved toward the volatiles of leaves that were slightly....
infested with conspecifics than toward the control leaves, and there was no significant difference in their movement toward volatiles of moderately infested and uninfested leaves (Fig. 1A). There was a significant difference in the attraction of *T. urticae* to leaves with different levels of conspecific infestation (contingency table analysis, chi-square = 10.898, d.f. = 2 and \( p < 0.01 \)). Among the three experiments, there was a significant difference between the response of *T. urticae* to slightly infested leaves vs. uninfested leaves and to heavily infested leaves vs. uninfested leaves (2×2 contingency table analysis, chi-square = 10.758, d.f. = 1 and \( p < 0.01 \)), and a marginally significant difference in the response to the slightly infested leaves vs. uninfested leaves and the moderately infested leaves vs. the uninfested leaves (2×2 contingency table analysis, chi-square = 3.839, d.f. = 1 and \( p = 0.0501 \)).

*T. urticae* showed no differences in movement between the volatiles from conspecifics (100 or 1,500 females), their associated products and uninfested leaves vs. uninfested leaves [100 females: 38 vs. 43 (no choice = 1); 1,500 females: 32 vs. 38 (no choice = 0), respectively]. There was also no difference in movement between the volatiles from leaves that were slightly or heavily infested with *S. exigua* and the volatiles from control leaves (Fig. 2A), and no significant difference in the response of *T. urticae* to the leaves at different infestation levels (2×2 contingency table analysis, chi-square = 0.029, d.f. = 1 and \( p = 0.866 \)).

**Responses of *P. persimilis* to the volatiles from *T. urticae* or *S. exigua*-infested leaves**

Significantly more *P. persimilis* moved toward volatiles from leaves that were heavily or moderately infested with *T. urticae* than toward those from the control leaves, but there was no difference in movement between the volatiles of slightly infested leaves and those of the control (Fig. 1B). There was a significant difference in the attraction of *P. persimilis* to leaves with different levels of *T. urticae* infestation (contingency table analysis, chi-square = 10.898, d.f. = 2 and \( p < 0.01 \)). Among the three experiments, there was a significant difference between the response of *P. persimilis* to the slightly infested leaves vs. uninfested leaves and the moderately infested leaves vs. uninfested leaves (2×2 contingency table analysis, chi-square = 6.733, d.f. = 1 and \( p < 0.01 \)), and a marginally significant difference between the response to the slightly infested leaves vs. uninfested leaves and the heavily infested leaves vs. uninfested leaves (2×2 contingency table analysis, chi-square = 3.976, d.f. = 1 and \( p = 0.046 \)). Moreover, there was no difference in *P. persimilis* movement between the volatiles from leaves that were either slightly or heavily infested with *S. exigua* and those of the control (Fig. 2B; 2×2 contingency table analysis, chi-square = 0.206, d.f. = 1 and \( p = 0.650 \)).

**Chemical analysis of volatiles**

The relative ratio of the total signal values of volatiles from the leaves that were slightly,
moderately and heavily infested with *T. urticae* was 3.20, 12.1, and 23.4 times more than that of the control leaves (Fig. 3). The amount of volatiles from heavily infested leaves was significantly higher than from uninfested leaves and slightly infested leaves (Fig. 3).

The relative ratio of the total signal values from the leaves that were slightly and heavily infested with *S. exigua* was 3.00 and 21.2 more times than the uninfested leaves (Fig. 3). The amount of volatiles from heavily infested leaves was significantly higher than from uninfested leaves and slightly infested leaves (Fig. 3).

Ten compounds, 

- (Z)-3-hexenyl acetate,
- (Z)-\( \beta \)-ocimene,
- (E)-\( \beta \)-ocimene,
- (Z)-4,8-dimethyl-1,3,7-nonatriene [(Z)-DMNT],
- linalool,
- (E)-4,8-dimethyl-1,3,7-nonatriene [(E)-DMNT],
- (E,E)-4812-trimethyl-1,3,7,11-tridecatetraene [TMTT],
- methyl salicylate,
- \( \beta \)-caryophyllene and
- \( \alpha \)-copaene were identified in the *T. urticae*-infested leaf volatiles (Fig. 4A). The compounds emitted from *T. urticae*-infested leaves (except for linalool) were also emitted from *S. exigua*-infested leaves (Fig. 4B). As the infestation level of *T. urticae* increased, so did the emission of the five volatile compounds [(Z)-3-hexenyl acetate, (E)-\( \beta \)-ocimene, (Z)-DMNT, (E)-DMNT, and TMTT] (Fig. 4). Similarly, as the infestation level of *S. exigua* increased, so did the emission of the above five volatiles plus (Z)-\( \beta \)-ocimene (Fig. 4).

Ozawa et al. (2000) reported 9 and 14 volatile...
compounds from *T. urticae*-infested and *S. exigua*-infested leaves, respectively. Similarly, Dicke et al. (1999) reported 52 volatile compounds, and Shimoeda et al. (unpublished) reported 21 compounds from *T. urticae*-infested leaves. The major compounds in Dicke et al. (1999) are the same as those found in this study, whereas most of their minor compounds were under the detectable level. This difference can be explained by the different experimental conditions, including the duration of infestation, number of herbivores, volatile sampling method [tenax adsorption (this paper) vs. SPME absorption (Ozawa et al., 2000)] and GC-MS apparatus.

**DISCUSSION**

There was a significant effect of female *T. urticae* density on the olfactory responses of female conspecifics to infested leaves over uninfested leaves, as summarized in Table 1. Dicke (1986) showed that, in a vertical olfactometer, *T. urticae* dispersed from volatiles emitted by *T. urticae*-infested lima bean leaves but walked tortuously when the infested-leaf volatiles were added to uninfested-leaf volatiles. The ratio of infested-leaf volatiles to uninfested-leaf volatiles also appears to inform the spider mites the relative level of infestation. Dicke et al. (1993) suggested that linalool was one of the compounds responsible for dispersal. In the present study, the highest emission of linalool was from leaves that were heavily infested with *T. urticae* (Fig. 4A), which may in part explain the negative response of *T. urticae* to this treatment.

There was a significant effect of herbivore density on the degree of *P. persimilis* attraction to the infested leaves when compared with uninfested leaves. The predators appear to prefer leaves with a large number of prey mites (Maeda and Takabayashi, 2001). Sabelis et al. (1984) reported that the response of *P. persimilis* to volatiles from leaves infested with *T. urticae* of all feeding stages, except for females, was significantly positive when their numbers were sufficiently large.

Leaves that are heavily infested with conspecifics are probably not a suitable food resource for foraging *T. urticae*, as a plant with such an infestation is likely to die rapidly. Further, such plants can be an enemy-dense patch for *T. urticae*, because *P. persimilis* are attracted to heavily *T. urticae*-infested leaves. Other predators, such as *Scolothrips takahashi* (Shimoda et al., 1997), *Amblyseius womersleyi* (Maeda et al., 1999) and *Oligota kashmirika benefica* (Shimoda and Takabayashi, 2001) also respond to *T. urticae*-infested lima bean leaf volatiles. Thus, foraging *T. urticae* should benefit by avoiding volatiles from heavily conspecific-infested leaves in terms of both resource availability and avoiding enemy-dense patches.

*P. persimilis* showed no difference in preference between volatiles from slightly *T. urticae*-infested leaves and uninfested-leaves. It is of interest that *T. urticae* individuals were attracted to volatiles from slightly *T. urticae*-infested leaves over uninfested-leaves, albeit there was no significant difference between the compounds found in slightly infested and uninfested-leaf volatiles. A small amount of (E)-β-ocimene and (E)-DMNT emitted from slightly infested leaves may be a signal for *T. urticae*, indicating available food resources and a patch where predators are not present.

*T. urticae* and *P. persimilis* did not show a significant preference between *S. exigua* infested and uninfested leaves (Fig. 2). The total emissions from heavily and slightly *S. exigua*-infested leaves were not different from those emitted by the same level of *T. urticae*-infested leaves (Fig. 3). Although (E)-β-ocimene and (E)-DMNT, which have already been reported to attract *P. persimilis* (Dicke et al.,

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<tr>
<th>Leaves infested with <em>T. urticae</em></th>
<th>Leaves infested with <em>S. exigua</em></th>
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<td>Slightly</td>
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<td><em>P. persimilis</em></td>
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<td><em>T. urticae</em></td>
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Table 1. Summary of the olfactory responses of *P. persimilis* and *T. urticae* to volatiles from herbivore-infested leaves vs. uninfested leaves in the Y-tube olfactometer.
were two major compounds in the volatiles of *S. exigua*-infested leaves, such leaves did not affect the olfactory response of *P. persimilis*. *T. urticae* and *P. persimilis* did discriminate between the blend of heavily *T. urticae*-infested leaf volatiles and that of heavily *S. exigua*-infested leaf volatiles. Shimoda and Dicke (2000) reported that *P. persimilis* were attracted to volatiles from lima bean plants infested by *S. exigua* larvae (two first-instar larvae per leaf in 3 d) when starved for 1 h but not when starved for 24 h. Thus, the response of *P. persimilis* in this study was also affected by their starvation condition.

This study showed that both starved *P. persimilis* and satiated *T. urticae* showed different olfactory responses to volatiles emitted from leaves infested by different numbers of conspecific or heterospecific herbivores (Table 1). As the duration of starvation also affects the response of *P. persimilis* (Shimoda and Dicke, 2000), the effects of the induced volatiles are even more complicated than we show here. Such multifunctional aspects of volatiles from both infested and uninfested plants may be an important factor affecting the dynamic community structures of herbivores and carnivores on plants.

**ACKNOWLEDGEMENTS**

We thank Takashi Ohbayashi for providing us with *S. exigua* eggs and Dr. Takayuki Mitsunaga for comments about the statistical analysis. This study was supported by CREST of JST (Japan Science and Technology Corporation).

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